

Retrospectives: Intersection of spaceflight stressors and microbial risk to crew and craft

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2022 NASA [Human](#) Research Program Investigators' Workshop



Overview

- Retrospectives on the road to risk reduction
- Spaceflight stressors in scope
- Flight-relevant microbial populations
- Microbiome of the built environment
- Host-microbe interactions
- Conclusions and recommendations



Risk reduction roadmap



Iterative process requiring periodic evaluation and progress assessments

- Micro-102: Evaluate whether deep-space radiation has an additive or synergistic effect with weightlessness /isolation/ confinement on microbial types, numbers, and virulence.
- Micro-103: Evaluate whether atmospheric composition (for example, elevated CO₂ levels) is a significant contributor to changes in the microbial profile of spaceflight.



Stressors: ionizing radiation

Property of the spaceflight environment

Photon: e.g., x-ray, gamma-ray

Particle: e.g., GCR

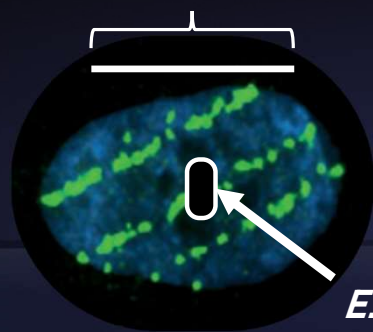
γ -ray

Si
Z=14

Fe
Z=26

Direct effect of radiation

$\sim 7\ \mu\text{m}$



E. coli cell
 $\sim 1\ \mu\text{m} \times 2\ \mu\text{m}$

Indirect effect of radiation

Ionizing radiation

H_2O

ROS
generation

H_2O_2

$\text{HO}\cdot$

$\text{HO}_2\cdot$

\vdots

Cellular
damage

DNA
Protein
Lipids

*How does IR affect flight-relevant
microbial populations?*

Cucinotta and Durante, Lancet Oncol 2006; 7: 431-35

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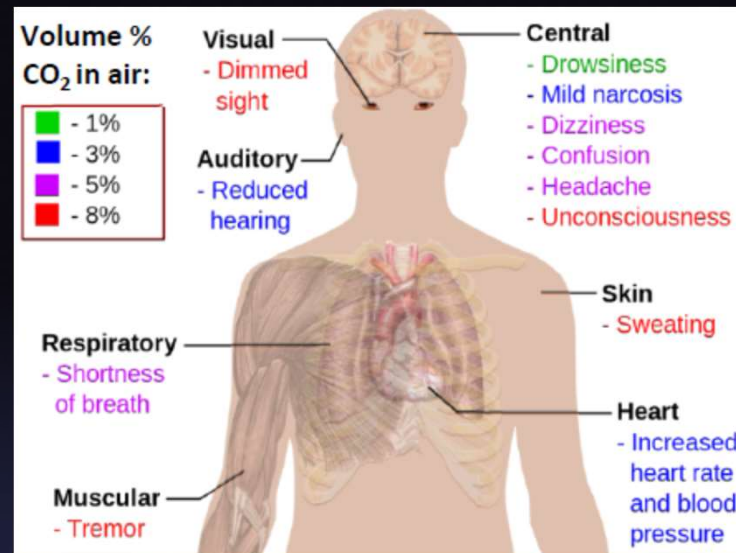
Stressors: elevated CO₂



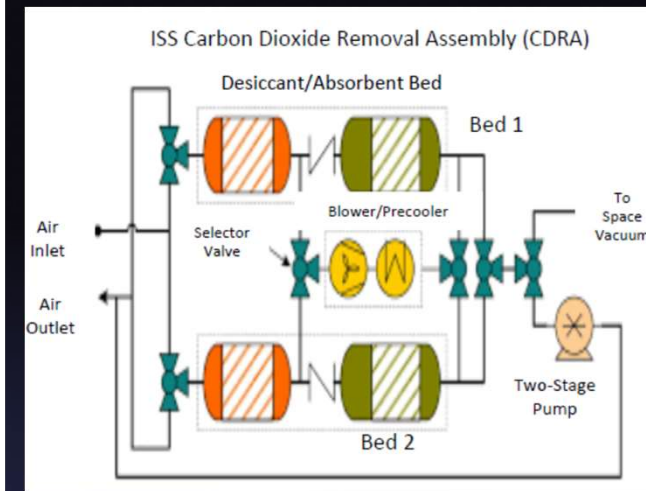
CO₂



Spaceflight Maximum Allowance Concentration (SMAC):
0.4% (3 mm Hg) CO₂ (24-hour avg)



Chemical removal of CO₂



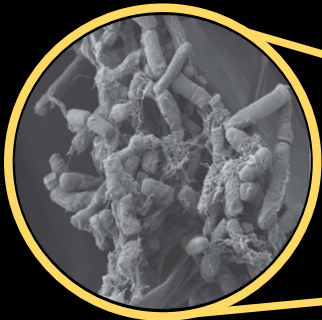
NASA-STD-3001 Technical Brief *Carbon Dioxide (CO₂)*
V2: 4015; 6001; 6004; 6020-22; 7041; 11034-39

How does elevated CO₂ affect flight-relevant microbial populations?



Flight-relevant microbial populations

Microbiome of the built environment (MoBE)



Consistently sampled
Concerns about crew health
Concerns about craft systems

Image: Mora, M. *et al. Nat Comm* 10, 3990 (2019)



Image credit: NASA

Host-microbe

Highly complex interactions

Multiple host tissues with their own microbiomes

Includes non-crew hosts, such as plants

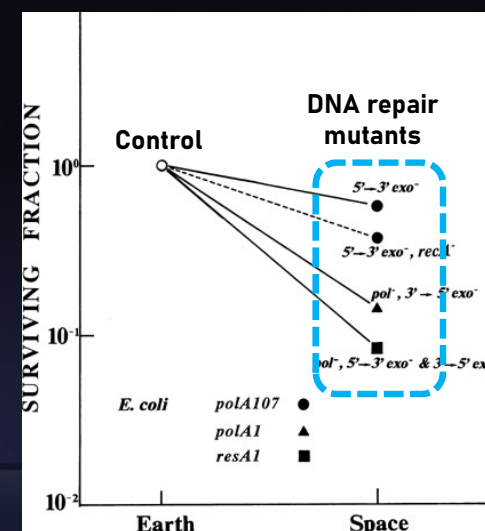
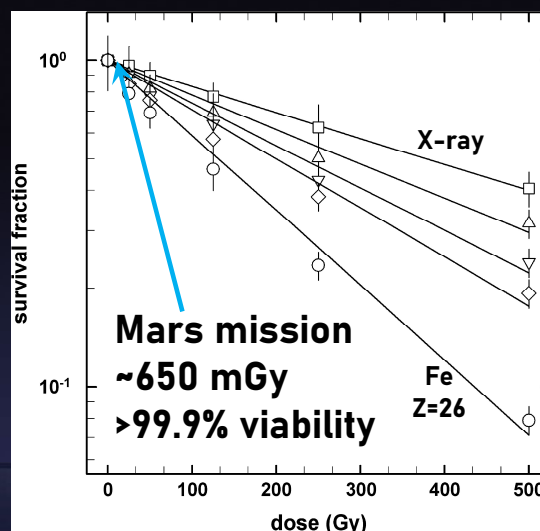
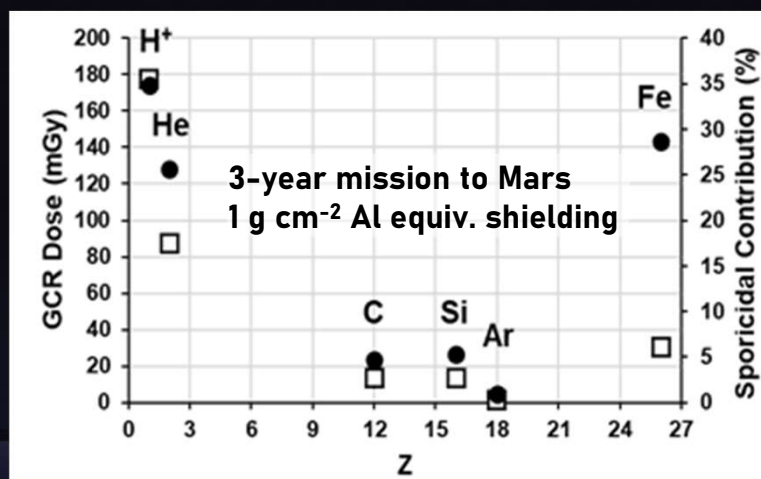


Effect of stressors on microbial risk

What is known about the univariate effect of these stressors on microorganisms?

Hierarchy of IR protection

DNA repair > physical protection (e.g., sporulation) > ROS detox



Adapted from:
Straume, et. al. Life Sciences in Space Research, 2017, 13:51-59

Moeller, et. al. Astrobiology. Jun 2010.509-521. Harada, et. al., FEMS Micro Letters, 1998 164:1, 39-45



Effect of stressors on microbial risk

What is known about the univariate effect of these stressors on microorganisms?

Increased $p\text{CO}_2$

Phototrophs

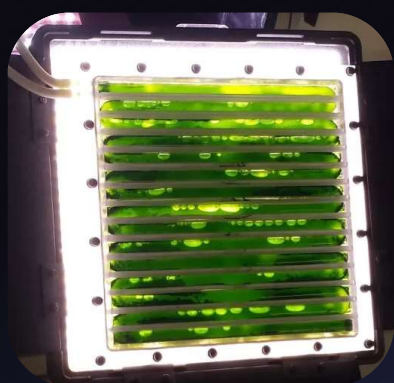


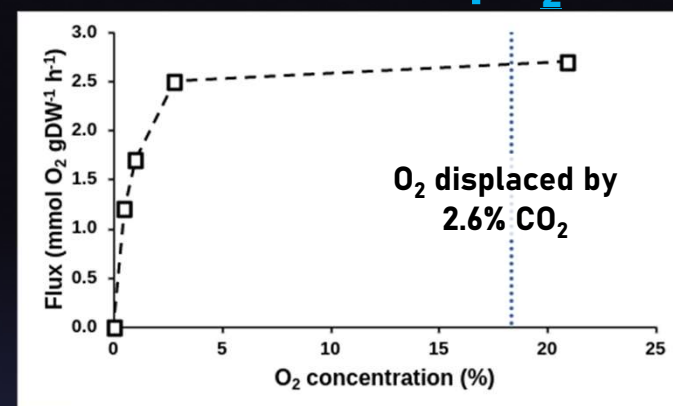
Image credit: ESA

Heterotrophic microbes

E. coli
Enterococcus faecalis } No fitness defect at 13% CO_2

Staphylococcus aureus } Fitness defect at 7% CO_2
But maintained virulence

Decreased $p\text{O}_2$



Adapted from:
Jouhten *et al.*, *BMC Syst. Biol.*, Jul. 2008, 2:1

There is not a ubiquitous hypercapnic microbial phenotype



Microbiome of the spaceflight built environment



MoBE-Retrospective framework

Separated into host- and spacecraft-associated microbiomes
Defined an ideal “end-state”

1. The source of microbial populations
2. The viable fraction of the population
3. The taxonomic and functional diversity
4. The duration/frequency of active vs. inactive metabolic periods
5. The relative power of CO₂/radiation stress versus other spaceflight stressors

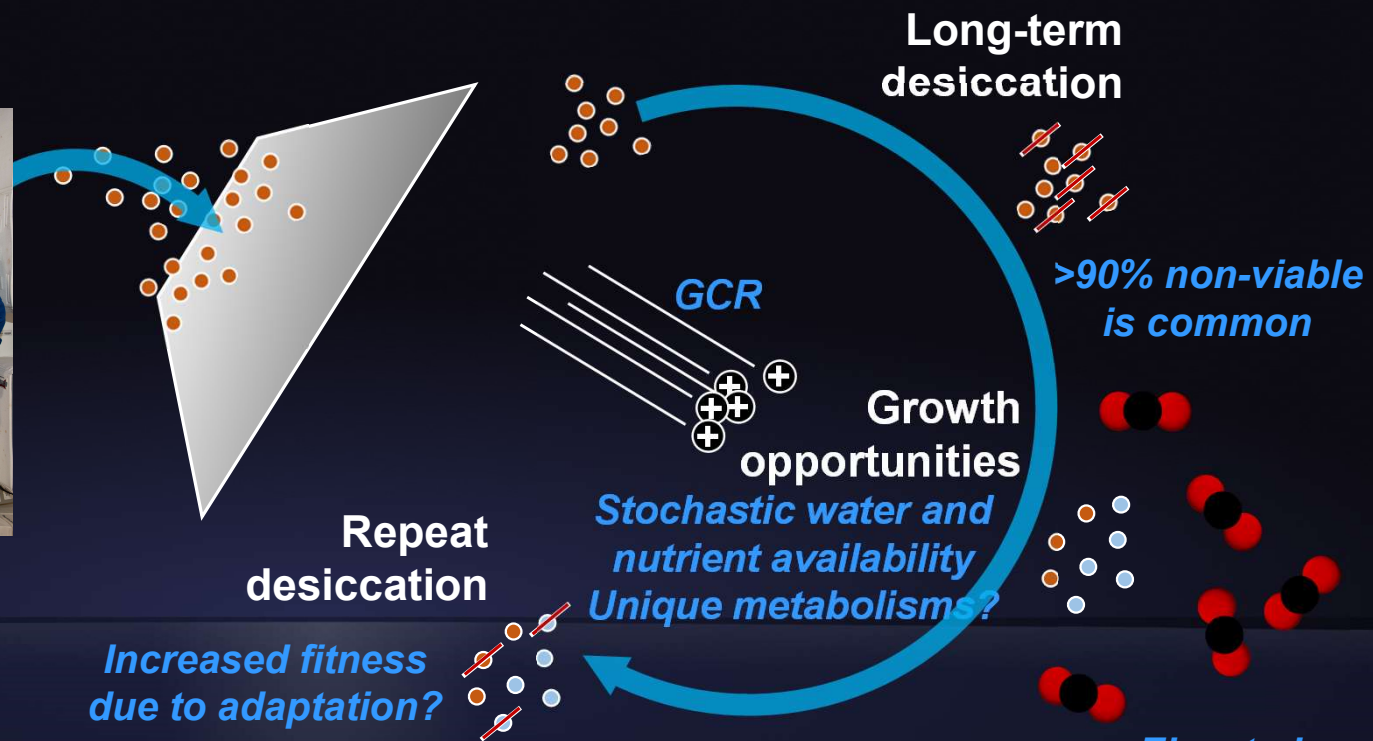


Source and viability of microbial populations

Image credit: NASA

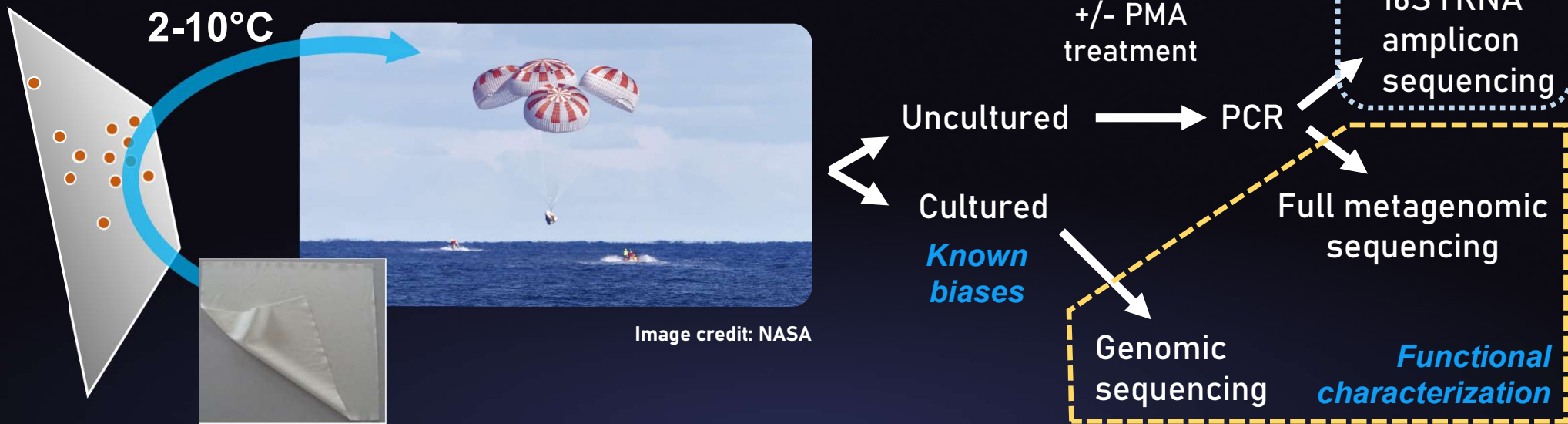


Source of spacecraft surface-associated microbes



Source and viability of microbial populations

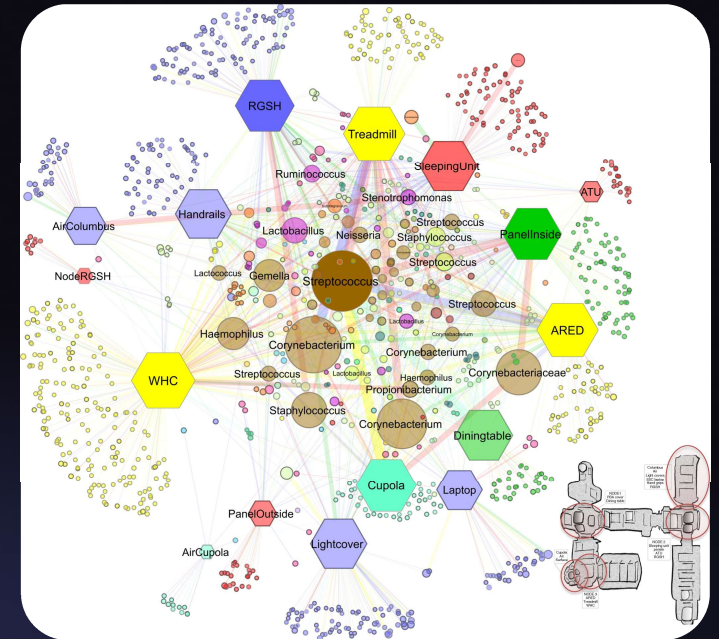
Current microbial monitoring process on the ISS



Cultured microbial genomes and 16S sequencing dominate current analysis
Currently funded projects are focused on full metagenomic sequencing

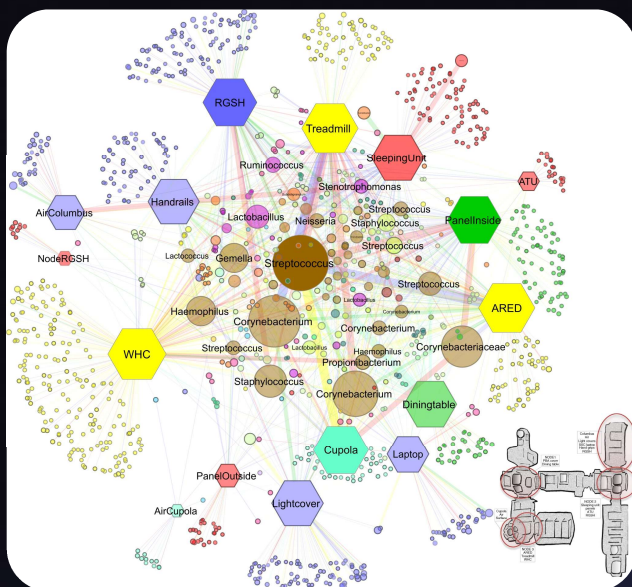


The crew is a dominant source of surface-associated microbial populations

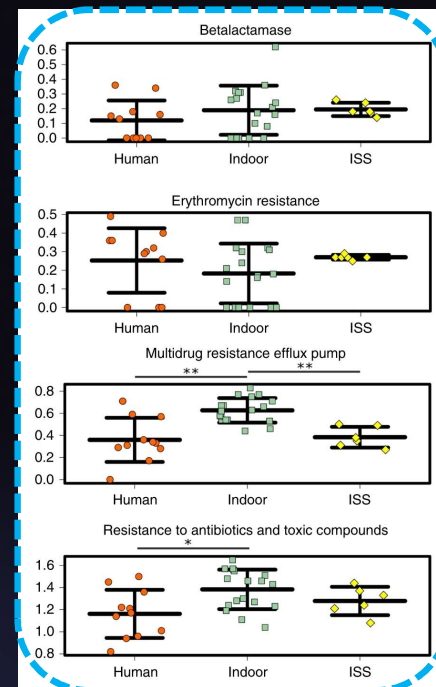
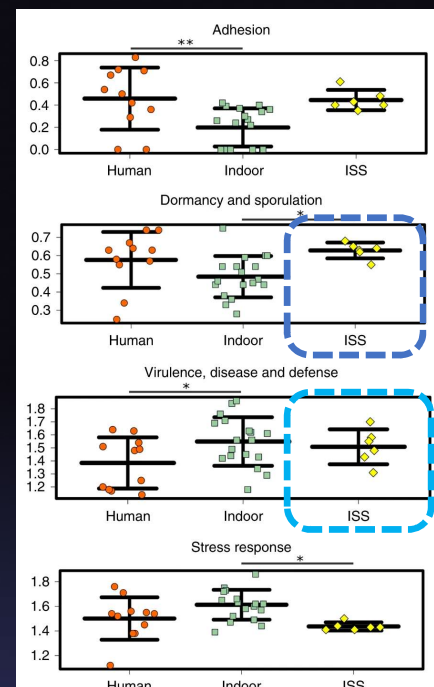
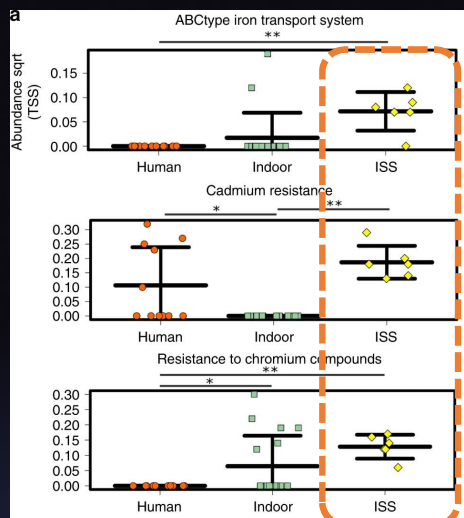


Mora, M., *et al. Nat Commun* **10**, 3990 (2019)

Taxonomic and functional diversity



Mora, M., *et al. Nat Commun* 10, 3990 (2019)

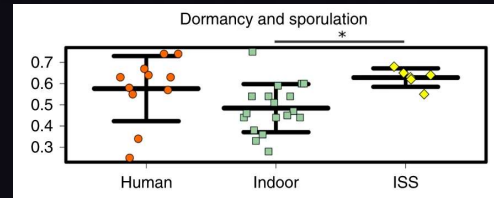
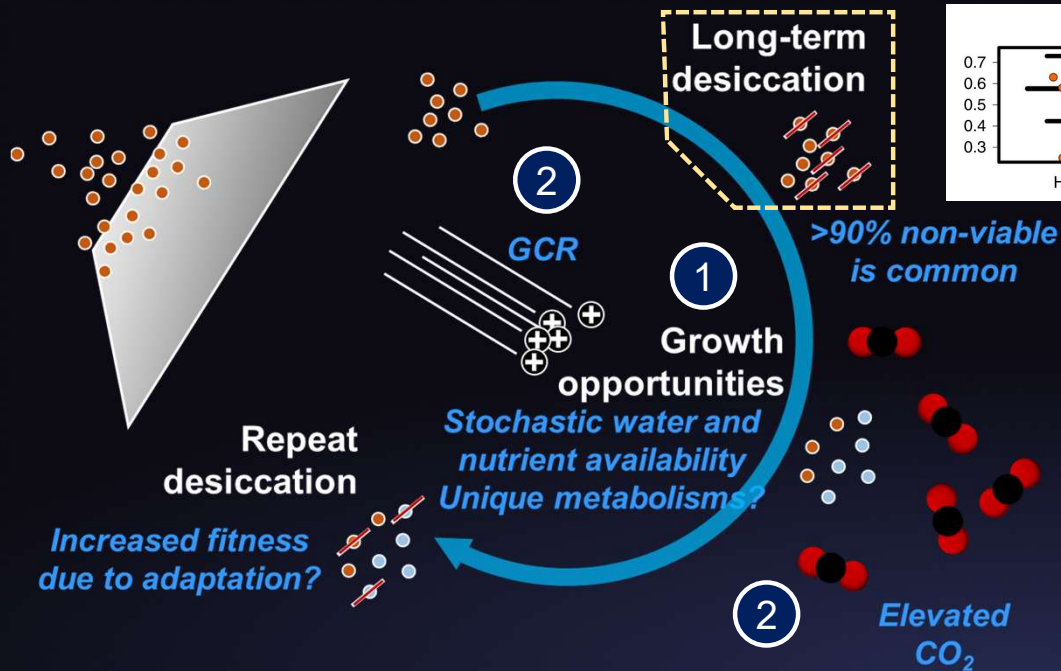


Functional enrichment analysis is sparse

Current data suggests enrichment in general built environment persistence



Active metabolism and relative stressors



1 Continued adaptation is predicated on increasing genetic diversity of the population through growth

Growth opportunities likely rare and random

2 The relative contribution of ionizing radiation and elevated CO₂ compared to desiccation on microbial selection is unknown



MoBE- Summary

1. The **source** of microbial populations

The crew microbiome is the dominant source

2. The **viable** fraction of the population

Somewhat ambiguous, but skin microbes appear to have a fitness advantage

3. The **taxonomic** and **functional** diversity

Taxa associated with the skin microbiome are enriched

Functional assessments are nascent, but suggest metal detox and resistance to dormancy and starvation are enriched

4. The duration/frequency of **active vs. inactive** metabolic periods

Poorly characterized, but active metabolic periods are hypothesized to be rare

5. The **relative power** of CO₂/radiation stress versus other spaceflight stressors

Unknown



Host-microbiome interactions in spaceflight



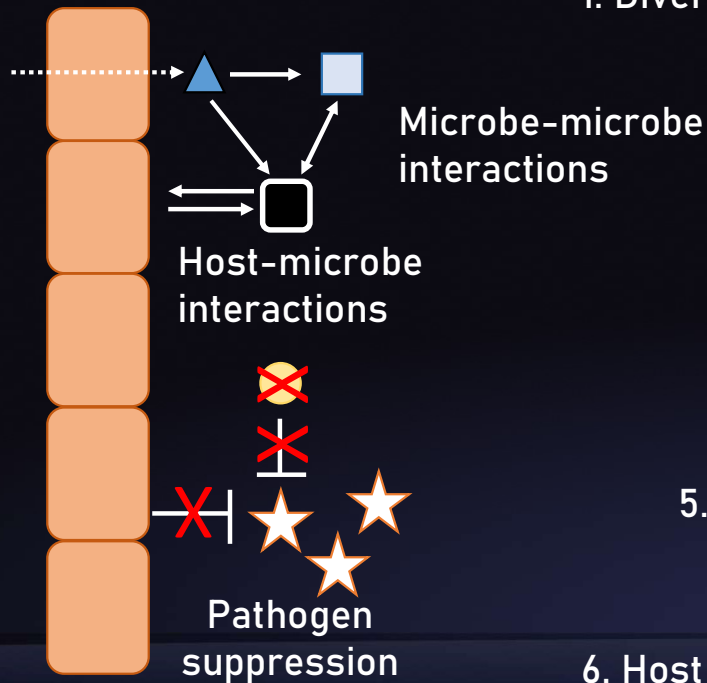
Host-microbe-Retrospective framework

1. The diversity of microbial populations in each *tissue*
2. The functional role of different taxonomic groups
3. The mechanistic interaction with the host tissue
4. The effect of elevated CO₂/IR on these microbes and the host-microbe interaction
5. The resilience of the microbiome community composition and function to maintain homeostasis
6. The effect of elevated CO₂/IR on the host and its reciprocal impact on the microbiome



Illustrative framework

Host tissue

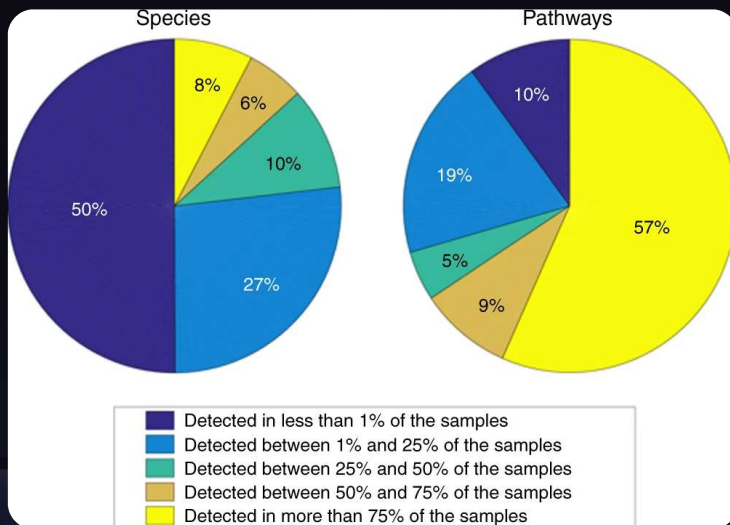


1. Diversity: **what is basal population and population variability between hosts?**
2. Functional role: **how does each microbe help maintain, or degrade, homeostasis?**
3. Mechanistic interaction with the host: **what is the reciprocal dependencies that maintain functional homeostasis?**
4. Stress effects: **How do stressors disrupt equilibrium?**
5. Resilience: **Is there functional redundancy in the system to re-establish functional homeostasis?**
6. Host effects: **How do perturbations in the host system affect the mechanistic interaction with the microbiome?**



Gut microbiome

16S rRNA sequencing is the norm
High taxonomic variation between individuals¹
Functional variability is much lower



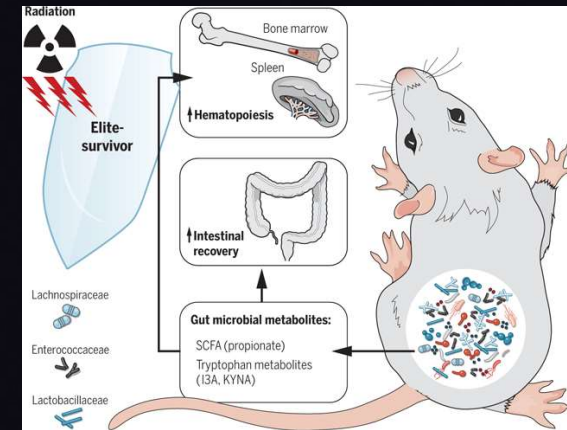
Visconti, A., *et al. Nat Commun* 10, 4505 (2019).

¹Vujkovic-Cvijin, *et al. Nature* 587, 448–454 (2020).

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Most data is from acute radiation doses
Host tissue is the likely target of damage



Guo H, *et al. Science*. 2020;370(6516):eaay9097

Ionizing Radiation

Elevated CO₂

Hypoxia/hypercapnia reduced intestinal barrier function

Links between gut microbiome and obstructive sleep apnea (OSA)

Conflicting data on the effect of inspired gas composition on circulating vital gas values

↑ IR

↑ Lachnospiraceae

↑ OSA²

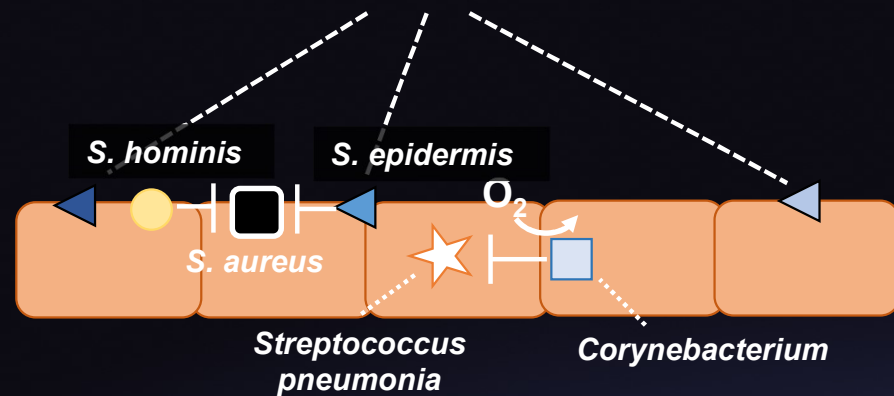
↑ flight³

²Tripathi A, *et al. mSystems*. 2018 Jun 5;3(3):e00020–18.

³Voorhies, A.A., *et al. Sci Rep* 9, 9911 (2019).

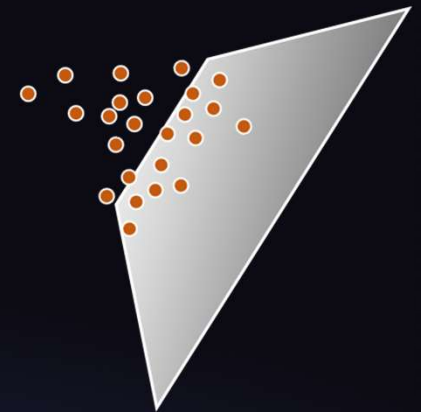
Skin microbiome

Site-specific strain heterogeneity



Flight studies are all 16S rRNA and often combine samples from multiple sites

Image credit: NASA



Studying the skin microbiome while still crew-associated may provide insights into MoBE populations



Conclusions and Recommendations

Conclusions

- MoBE:
 - Source of microbial populations is known
 - Relative impact of different stressors is unknown
- Host-microbe:
 - Characterizing flight dysbiosis has the same challenges as terrestrial studies

Recommendations

- 1) Focus on metagenomics, functional characterization, and predictive modeling
- 2) More realistic ground studies- e.g., inspired CO₂ and chronic IR exposure
- 3) Model systems such as tissues-on-a-chip and defined microbial communities

